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DESIGN AND DEVELOPMENT OF SPR INSTRUMENT BASED ON ϴ-2ϴ MECHANICAL INTEGRATION TECHNIQUE

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ABSTRACT

Surface Plasmon Resonance (SPR) instrument is an important tool in the field of biomolecular interaction studies. The SPR instrument is basically an optical measurement technique and its construction is very complex. The construction of the SPR instrument is not that much easy because it is an integration of optics, mechanical, electronics and electrical. However, the instrument operation depends on the mechanical movement of the resonance angle. Based on the mechanical aspects the instrument is constructed in different aspects for better generation of SPR signals. To generate SPR signal the optical prism with a sensor is rotated by the stepper motor for resonance angle and the signals are collected at the detector. The optical setup is rotation by ϴ-2ϴ for optimal to produce the effective SPR signals. This paper describes the fabrication and working of SPR instrument based on ϴ-2ϴ.

***KEYWORDS:** Surface Plasmon Resonance (SPR) Instrument, Prism, Laser Diode, Stepper Motor, ϴ-2ϴ & PIC Microcontroller*

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Original Article

INTRODUCTION

There is no much instrument available in the world for molecular interaction studies. Among the few instruments, Surface plasmon resonance is a highly sensitive instrument [1, 2]. The cost of the surface plasmon resonance instrument is very high due to an involvement of optical components and complex construction [3]. A high power monochromatic laser beam is directed over optical prism, at resonance angle, the totally internally reflected beams collected by photodetectors [4, 5]. The signals from the photodetector are signal processed by the embedded controller [6]. The optical prism is moved by a stepper motor for a wide angle so as to generate the SPR signal. The signal generated is captured by the photo-detector for measurement of the signal. The possible methods tried are,

- **Experimental Set up I:** Moving laser source, stationary optical Prism, and photodetector
- **Experimental Set up II:** Moving optical prism, photodetector and stationary laser source
- **Experimental set up III (ϴ-2ϴ):** Moving optical prism, moving Photodetector and stationary laser source

The instrument is constructed and fabricated by using all the three experimental setup. Among them, experimental setup III (ϴ-2ϴ) is a better method for generating SPR signals. This paper describes the various experimental setup construction and fabrication of SPR instrument.

METHODS AND MATERIALS

Surface Plasmon resonance is generated by three methods, among them kretschmann is most popular and optimal method of generating. Based on the kretschmann configuration all the experimental methods are developed. The kretschmann based experimental setup consists of an optical prism, gold-coated glass plate, 10KW monochromatic laser beam, photodetector. Since the instrument is an optics based instrument, prism and laser beam is involved; the surface plasmon signal generated is totally on the total internal reflection.

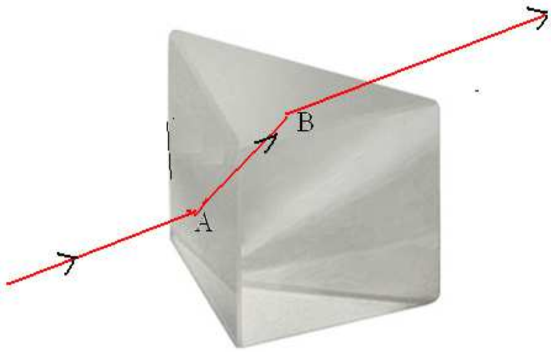

Figure 1: Kretschmann Based SPR Setup, Laser Beam Passed Below the Critical Angle

The figure 1 shows the laser beam is passed through the prism at an angle less than the critical angle and laser beam gets refracted through the prism, SPR signal is not produced.

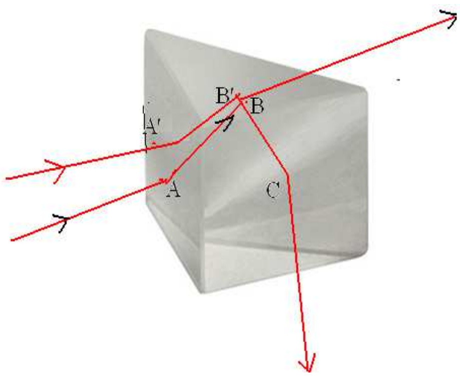

Figure 2: Kretschmann Based SPR Setup, Laser Beam Passed above the Critical Angle

The figure 2 shows the laser beam is moved above the critical angle, due to total internal reflection the SPR signal is generated. It is observed the laser beam should be moved above the critical angle to generate the SPR signal. Either the optical prism with sensor or laser beam should be moved by the programmed stepper motor. The generated signal collected by the photodetector measured in digital oscilloscope for further analysis. The design and fabrication of the SPR instrument are described below by different experimental setup. The optimal method is obtained through the development of the different prototype.

Moving Laser Source, Stationary Optical Prism and Photodetector- Experimental setup I

In this, the experimental setup the instrument is built such that the laser beam is rotated by the stepper motor and the laser beam graces over the hypotenuse of the prism to produce the SPR signal. The optical prism with gold-coated sensor is placed stationary and the laser beam is moved above the critical angle by the stepper motor. The figure 3& 4 shows the experimental prototype I. Prism is fixed horizontally and a laser beam is allowed to move over the gold coated prism surface back and forth. The laser diode is mounted over the stepper motor arm is rotated over the angle from 25 degrees to 65 degrees in steps back and forth. Since the stepper motors are all having 200 steps per revolution, at each step the angle change will be 1.8^{0}. The laser source is moved by using a programmed PIC 16F73 microcontroller. The SPR generated is collected at the solar panel and measured in the digital oscilloscope.

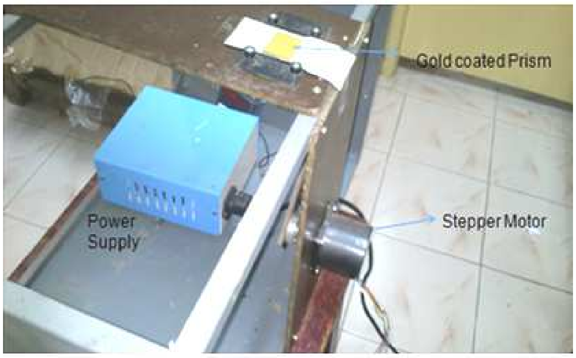

Figure 3: Experimental Prototype of SPR Instrument, Fixed Prism and Rotating Laser Source

We could not achieve the desired signal resolution in this moving source set up. The weight of the moving arm could not let fine angular movements to be made. This requires more powerful stepper motor. Hence we dropped this setup.

Figure 4: Experimental Prototype of SPR Instrument, Fixed Prism and Rotating Laser Source

MOVING OPTICAL Prism, Stationary Laser Source, and Photo Detector-Experimental Setup II

Due to the drawbacks of moving laser source method, the new technique is tried with a fixed laser source and a rotating prism. The figure shows the optical prism with sensors is placed over the turning table with a gear ratio of 1:30.

Here we have used high power He-Ne laser beam is used instead of the laser diode to produce a dense SPR signal. It is possible to operate the He-Ne laser for a relatively long duration of about an hour continuously. Besides, it is required to rotate the laser gun so as to adjust the plane of polarization to be on the face of the hypotenuse of the prism. After turning the laser tube so as to get the SPR dip of the maximum value, which corresponds to the correct polarization. He-Ne laser beam is placed in the stand in stationary at a place to undergo total internal reflection by rotating the prism table. The figure 5 shows the experimental prototype of moving prism table and stationary laser source. The SPR generated by this method is stable and better than the experimental setup I method. However, the sensitivity of the instrument is important in biomedical filed, by selecting a good geared stepper motor the resolution can be improved in the sensing of molecular interactions in 0.001RU. The other drawbacks of this experimental setup is rotating setup is heavyweight, occupies more space due to this we cannot build a compact instrument. Due to sensitivity and to develop a compact instrument, ϴ-2ϴ SPR instrument is tried.

Figure 5: Experimental Prototype of SPR Instrument Rotating Prism and Fixed Laser Source

Moving Optical Prism, Moving Photodetector and Stationary Laser Source (ϴ-2ϴ)-Experimental Setup III

When the angle of incidence changes by $\delta\theta$, the reflected beam is changing by twice this value. If a small size detector like a photodiode or Light Dependent resistor is used, we have to move the detector by double the angle of the source beam. Only then, the detector can follow the reflected ray and give a signal based on the reflected ray. Such a ϴ-2ϴ movement is called Goniometer. Using such an angular movement for the detector arm will ensure that at all angles; the reflection ray will be falling over the detector.

We devised a belt based arrangement for getting the detector arm move at twice the angle of rotation of the prism table. Hence the prism table and the detector are connected with a belt to the stepper motor with a pulley ratio of 1:2. The prism table carries the prism and rotates while the detector rotates at twice the angle. The figure 6 shows the SPR design of ϴ-2ϴ arrangement.

The gear ratios of these two motors were 1:8 and 1:30. Thus, the angle resolution of the 1:30 gear motor is 1.8 deg/30 = 0.06deg per step. If micro stepping is done the resolution is double this value. This compares with commercial instruments of the high cost.

The prism with attached gold slide is turned as the table rotates. The adjacent flow cell carries a sample also turns and hence inlet and outlet connections are made through flexible silicone tubing. A sharp SPR curve could be generated by a Light Dependent Resistor (LDR) signal, with this ϴ-2ϴ arrangement. The reflected signal is measured across the LDR

and given to the Oscilloscope.

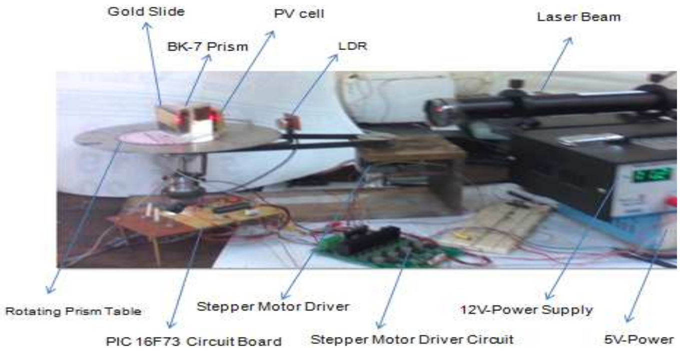

Figure 6: Experimental Prototype of SPR Instrument Rotating, Prism Rotating Detector and Fixed Laser Source

RESULT AND DISCUSSIONS

The SPR signal generated through ϴ-2ϴ technique is more sensitive than the other technique. From the experimental setup and simulation results, it is found the SPR generated is exactly the same and produced at the same angle with few noise signals added to the signal. This SPR signal noise can be removed by adding an amplifier and filter circuit. We have tested with samples of water and polluted water with the addition of few solutions to show how the SPR curve shifts sensitively. Thus the real-time plotting of SPR using PIC microcontroller and visual basic software has become feasible.

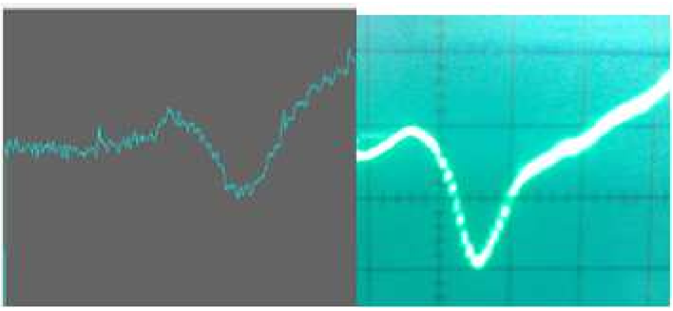

Figure 7: Experimental Prototype of SPR Instrument Rotating Prism, Rotating Detector and Fixed Laser Source

Besides DSO waveform observation, the signal from the PV cell through ADC of the microcontroller is plotted in real time in the Laptop screen is shown in figure 7. The LCD used in the microcontroller board displays the direction of the stepper motor, step value and the signal intensity of the PV cell plotted in the laptop.

CONCLUSIONS

A description of the total design and development carried out for the fabrication of the SPR with an economical budget and good enough sensitivity has been attempted. In short, all these designs have the following in common: The Glass Prism itself, the Source of Light, the angular variation of the beam of light, the photodetector and the arrangement of the Gold layer as well as the analyzed substance, usually in a flow cell. However, it is observed the experimental setup III is the best possible method of generating the SPR signals. Future work will be carried by replacing the LDR with a solar panel as the detector. Finally, an instrument was constructed as a semi-automatic instrument. The instrument is provided with a user-friendly computer interface as well. The shift of SPR curve due to change of the refractive index change can be

sensed by the plot on the computer.

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